Electronic Supplementary Material (ESI) for Analyst

Supporting Information

Revisit of a dipropargyl rhodamine probe reveals its alternative ion sensibility in both a solution and live cells

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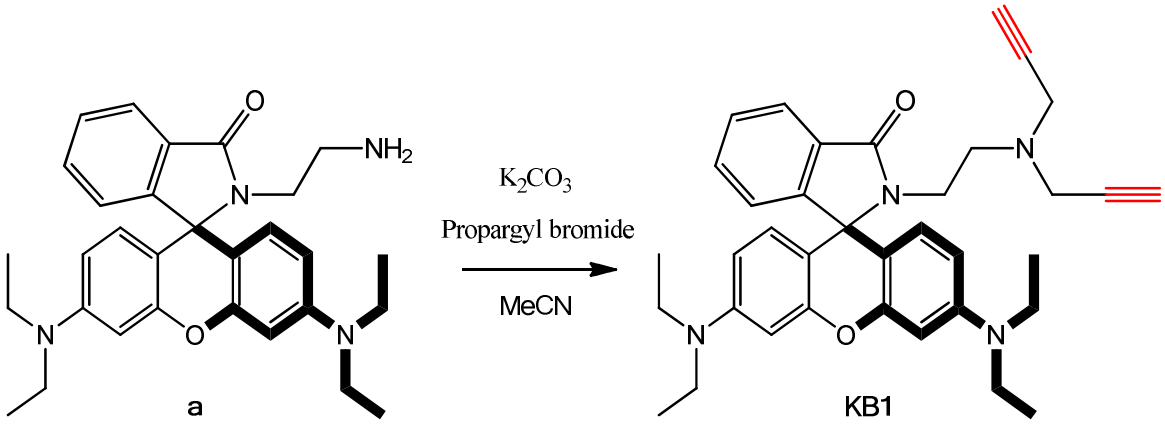
S1. Experimental section

General

All chemicals and regents are of high commercially available grade, and were used as received. ^{1}H NMR spectra were recorded on a Bruker AM-400 spectrometer using tetramethyl silane (TMS) as the internal standard (δ = 0). High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier XE spectrometer using standard conditions (ESI, 70 eV). All UV-vis absorption spectra were measured on a Varian Cary 500 UV-vis spectrophotometer and fluorescence spectra measured on a Varian Cary Eclipse Fluorescence spectrophotometer.

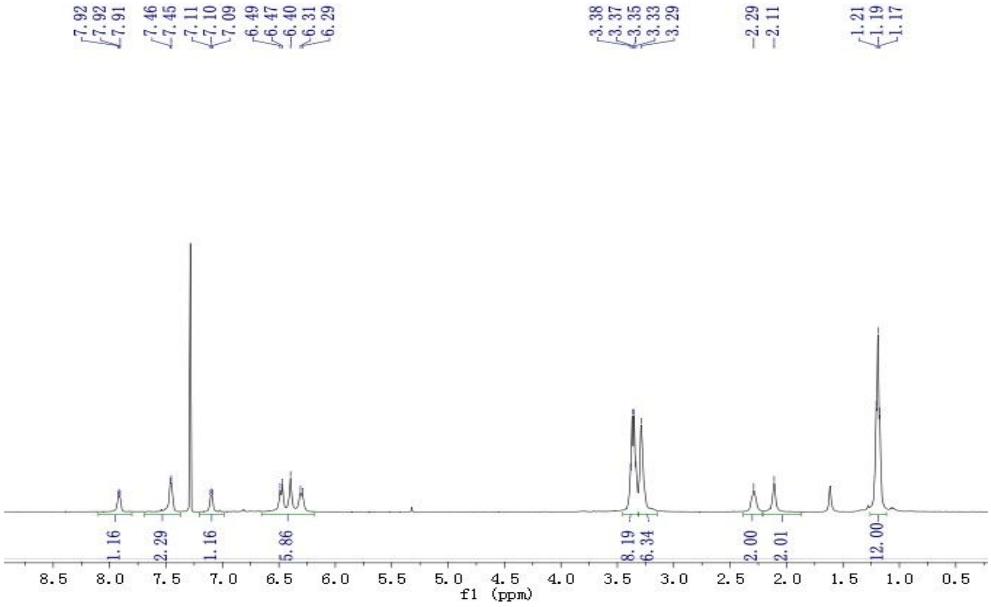
Synthesis of KB1



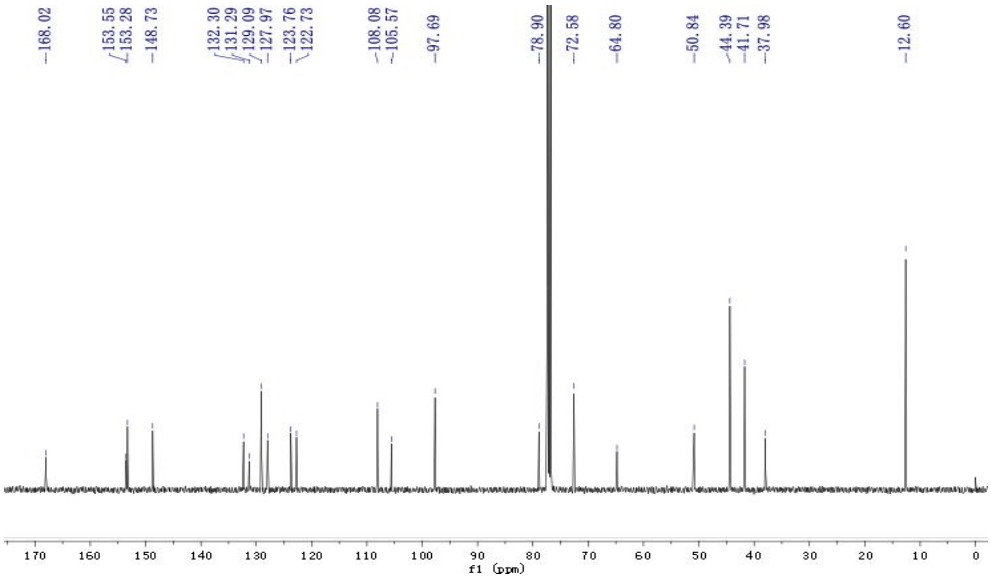
A mixture of **a** (1.2 g, 2.4 mmol), K_2CO_3 (0.5 g, 3.9 mmol) and propargyl bromide (0.18 mL, 2.4 mmol) in CH_3CN (20 mL) was stirred over night. Then the mixture was concentrated and washed with water and brine, and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered and concentrated in vacuum. The resulting residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 100:1, V/V) to afford **KB1** (500 mg, 37%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17-1.21 (t, J = 8 Hz, 12 H), 2.11 (s, 2H), 2.29 (t, J = 8 Hz, 2 H), 3.29 (s, 6 H), 3.33-3.38 (dd, J = 14 Hz, 8 H), 6.29-6.31 (d, J = 8 Hz, 2 H), 6.40 (s, 2 H), 6.47-6.49 (d, J = 8 Hz, 2 H), 7.10 (m, 1 H), 7.45-7.46 (m, 2 H), 7.91-7.92 (m, 1 H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 12.6, 38.0, 41.7, 44.4, 50.8, 64.8, 72.6, 78.9, 97.7, 105.6, 108.1, 122.7, 123.8, 128.0, 129.1, 131.3, 132.3, 148.7, 153.3, 153.6, 168.0. HR-ESI-MS m/z: $[M + H]^+$ calcd. for 561.3230, found 561.3166.

^{1}H NMR of **KB1**:



^{13}C NMR of **KB1**:



Spectroscopic measurements

Stock solution of **KB1** (500 μM) was prepared in CH_3CN. Stock solutions of 5 mM of $Cd(ClO_4)_2$, $Co(ClO_4)_2$, $Zn(ClO_4)_2$, $Ba(ClO_4)_2$, $Mg(ClO_4)_2$, $KClO_4$, $NaClO_4$, $Mn(ClO_4)_2$, $Pd(ClO_4)_2$, $Pb(ClO_4)_2$, $Ca(ClO_4)_2$, $Ag(ClO_4)_2$, $Ni(ClO_4)_2$, $Hg(ClO_4)_2$, $Cu(ClO_4)_2$, and 10 mM of Na_2S were prepared in deionized water.

The fluorescence measurements were carried out with a path length of 10 mm and an excitation wavelength at 530 nm by scanning the spectra between 540 nm and 750 nm. The bandwidth for both excitation and emission spectra was 5 nm. Unless otherwise mentioned, all the spectra were recorded in CH_3CN/H_2O (1: 1, V/V) at 25 °C.

Cell culture and transfection

Hep-G2 cells (cell bank, Shanghai Institutes for Biological Sciences) were maintained in a Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Gland Island, NY, USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C and split when the cells reached 90% confluency.

MTS cell viability assay

Hep-G2 cells were plated overnight on 96-well plates at 5000 cells per well in growth medium. After seeding, cells were maintained in growth media treated at increasing concentrations (6.25 μM, 12.5 μM, 25 μM, 50 μM, 100 μM and 200 μM) of **KB1** (dissolved in DMSO, final concentration) for 72 h. A 20 μL MTS (Promega Corp) solution (2 mg/mL) was then added to each well at 37 °C. After incubation for 2 h, the absorbance of each well was measured on a SpectraMax 340 microplate reader (Molecular Devices, USA) at 490 nm with a reference at 690 nm. The optical density of the result in MTS assay was directly proportional to the number of viable cells. Each experiment was done in triplicate.

Cell imaging

Hep-G2 cells were cultured in DMEM supplemented with 10% FBS. Cells (1.5×10^4/well) were seeded on a black 96-well microplate with an optically clear bottom (Greiner bio-one, Germany) overnight. After pretreatment with 20 mM of $Hg(ClO_4)_2$ or $Pd(ClO_4)_2$ in 50 mM Hepes for 30 min, the cells were incubated with the probe at different concentrations (10 μM or 25 μM) for another 30 min. Then the cells on the microplate were rinsed in warm Hepes and fixed by 4% paraformaldehyde for 15 min at room temperature. After three rinses in Hepes (5 min each time), the fluorescence was eventually detected and photographed with an Operetta high content imaging system (Perkinelmer, US).

S2. Fig. S1-Fig. S6

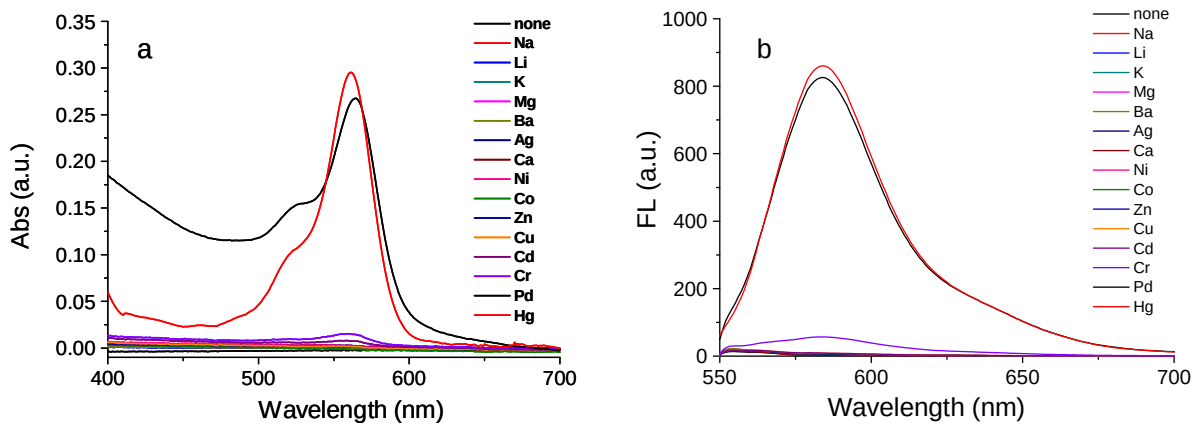


Figure S1. (a) UV-vis and (b) Fluorescence spectra (λ_{ex} = 530 nm) of 10 μM of **KB1** in the absence and presence of 50 μM of various metal cations in CH_3CN/H_2O (1: 1, V/V).

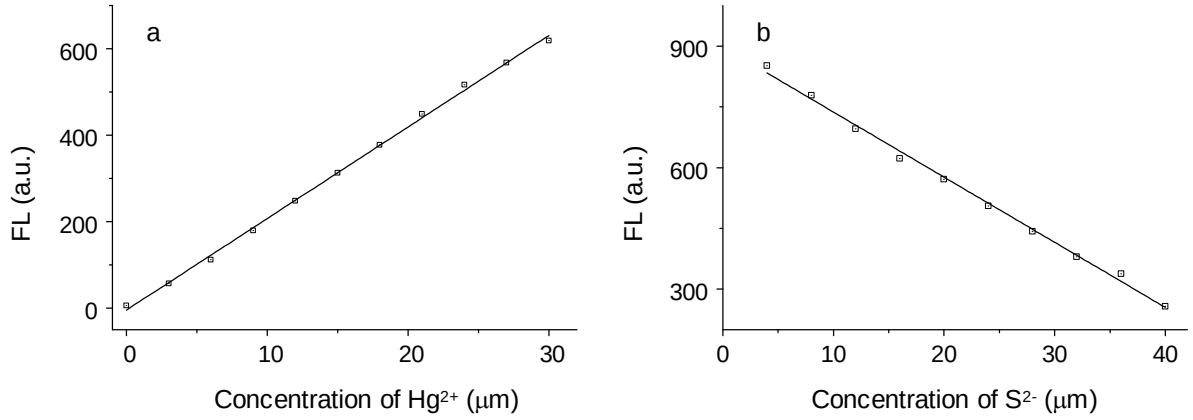


Figure S2. Plotting the fluorescence alternation of (a) **KB1** (10 μM in CH_3CN/H_2O = 1:1 [V/V]) as a function of Hg^{2+} concentration, and (b) **KB1** (10 μM in CH_3CN/H_2O = 1:1 [V/V])-Hg^{2+} complex as a function of S^{2-} concentration (λ_{ex} = 530 nm).

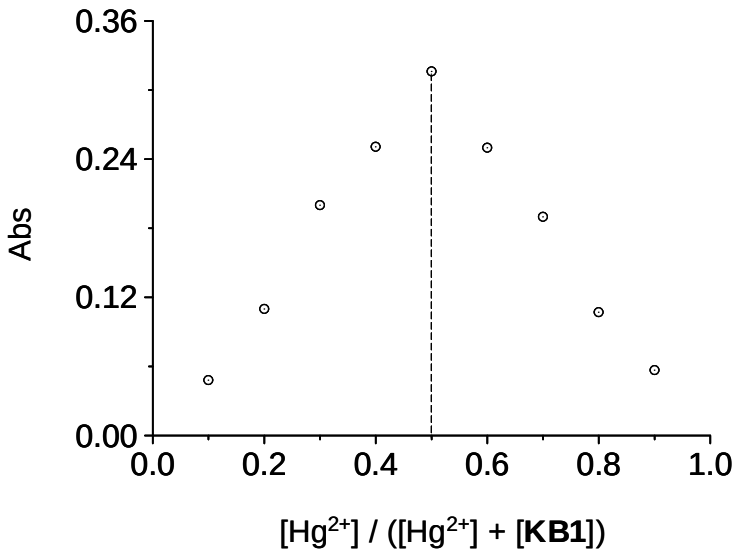


Figure S3. Job plot analysis of **KB1** (60 μM in CH_3CN/H_2O = 1:1 [V/V]) in the presence of Hg^{2+} with varying concentrations (λ_{ex} = 530 nm).

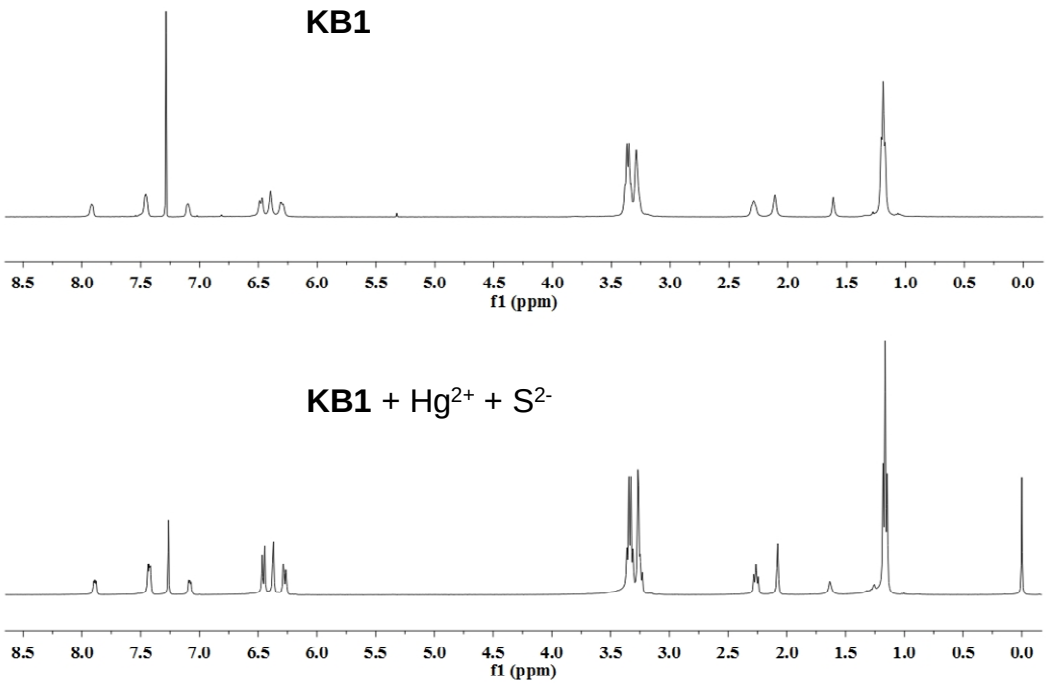


Figure S4. ^{1}H NMR spectra of **KB1** (upper) and **KB1** treated with Hg^{2+} and then S^{2-} (nether).

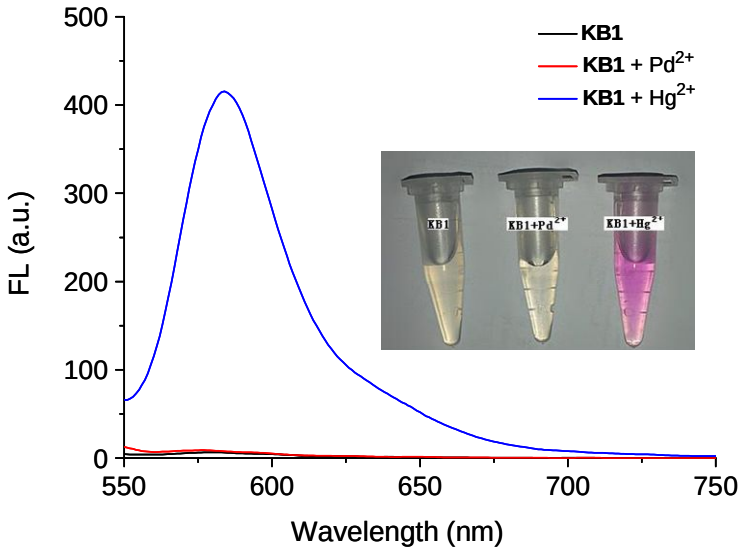


Figure S5. Fluorescence spectra of **KB1** (60 μM in Hepes/MeCN = 1:1 [V/V]) in the absence or presence of 600 μM of Hg^{2+} or Pd^{2+} (λ_{ex} = 530 nm).

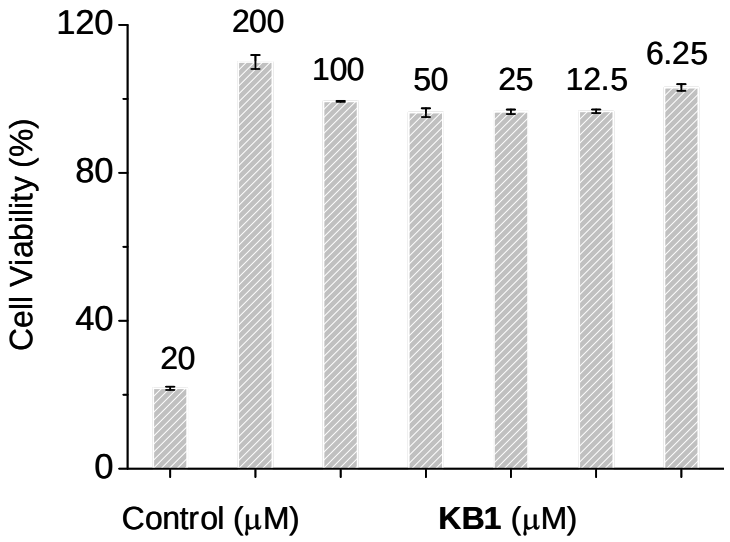


Figure S6. Hep-G2 cell viability in the presence of control (doxorubicin) or **KB1** with varying concentrations measured by MTS.